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Distribution of *CCR5 Δ32* HIV-1 resistance allele frequency at Zanjan province in Iran

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Abstract

Introduction: The CC chemokine receptor 5 (CCR5) is known as the main co-receptor in human immunodeficiency virus (HIV) infection. Accumulating evidence verified 32bp deletion in both alleles of *CCR5* provides natural resistance to HIV infection. Accordingly, recent therapeutic approaches are based on gene and cell therapies by inducing this resistance. Allogeneic transplantation of stem cells with *CCR5Δ32* homozygous to HIV infected people has been considered as an efficient strategy.
Objectives: The aim of this study was to determine *CCR5 Δ32* mutation frequency in healthy and HIV-infected individuals in Zanjan province, in Iran and also detecting appropriate candidate for future therapeutic approaches.
Patients and Methods: In this study, blood samples were collected from 102 HIV infected patients and 204 healthy controls in ethylene diamine tetra-acetic acid (EDTA) pre-coated tubes. DNA extraction was performed and analyzed for *CCR5Δ32* mutation by GAP-PCR in both groups and followed by 2% gel agarose electrophoresis. The *Δ32* genotype frequency was statistically determined.
Results: Our finding demonstrated that 3 HIV infected patients displayed *CCR5* mutation, while 204 healthy controls showed one *CCR5 Δ32/Δ32* homozygote mutation. We observed that *Δ32* mutation frequency is 0.82% in Zanjan province (χ^2 =47.58, $P<0.001$).
Conclusion: Reduction in *CCR5* mutation frequency as well as the other polymorphisms related to HIV resistance, can lead to the development of HIV infection epidemiology. Migration, natural selection and genetic admixture can elucidate various distribution of *Δ32* allele frequency in different populations, like as north Europeans that reduce gradually to south. The result of this study can facilitate the presentation of suitable *Δ32* donors to HIV infected patients in therapeutic approaches.

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Introduction

Human immunodeficiency virus (HIV) is a retrovirus that infects CD4⁺T cells and causes progressive failure of the immune system. After viral entry into the host immune cells, external virus glycoprotein (gp120) binds to CD4⁺ receptor and provides conformational change in gp41 that leads to interaction with the co-receptors (Cc-chemokine receptor-5: CCR5 or CXCR4). It has been demonstrated that 32bp deletion in both alleles of *CCR5* inhibits HIV entry into the CD4⁺ T cells, macrophages and provided "natural resistance" to HIV (1, 2). Therefore, CCR5 is known as a chief co-receptor in HIV infection (3, 4).

The significant limitations of conventional treatments as antiretroviral drugs, have led scientists to investigate more efficacious remedies as approaches based on gene and cell therapy (5, 6). One of the popular candidates for gene therapy is *CCR5*. Hematopoietic stem cell transplantation by *CCR5 Δ32/Δ32* genotype showed effective eradication of viral loading from a donor to HIV affected patient (7-9). Finding the suitable donor with HLA-matching and *Δ32* genotype is one of the problems in allogeneic transplantation. In addition, induction of natural resistance by Zinc finger nucleases artificially in hematopoietic stem cell exhibited successful results in HIV infected patient treatment (10). In addition to HIV, recent studies presented the key role of *CCR5* in therapeutic approaches of viral infection (11, 2).

As regards, no study has been conducted

Key point

In a study on 102 HIV-infected patients, we found reduction in *CCR5* mutation frequency as well as the other polymorphisms related to HIV resistance, can lead to the development of HIV infection.

in Zanjan province to evaluate the distribution of *CCR5 Δ32* allele frequency, therefore one of the target samples by homozygous *Δ32* genotype can be an appropriate candidate for HIV infected patients in Iran or other countries.

Objectives

The aim of this study was to determine CCR5 Δ32 mutation frequency in healthy and HIV infected individuals in Zanjan province, in Iran and also detecting appropriate candidate for future therapeutic approaches.

Patients and Methods

Study design

Specimens were collected from 102 HIV patients and 204 healthy controls in 4 mL ethylene diamine tetra-acetic acid (EDTA) pre-coated tubes (2 mL for DNA extraction and PCR, 2 mL for backup) at Zanjan province of Iran. The HIV infection was confirmed previously in patients by enzyme immunoassay (EIA) and western blotting. Both infected and healthy individuals were randomly selected. Informed consent was obtained from all the individuals that participated in this study. Demographic information of both study groups were analyzed by SPSS (version 16) software (Table 1).

DNA preparation

Genomic DNA was extracted by a commercial kit (QIAamp® DNA Mini kit, Cat. No: 51104, Germany) from whole blood samples. Then the DNA was aliquoted in -20°C for further using.

Gap-PCR reactions

The Gap-PCR was performed in a total volume of 25 µL by the following conditions; 12.5 µL of Master mix (Sinaclon, Cat. No: PR8251C, Iran), 1 µL of each primer, 2 µL of prepared DNA and 8.5 µL sterile deionized water. The sequences of the forward and reverse primers were respectively 5′CAAAAAGAAGGTCTTCATTACACC-3′ and 5′-CCTGTGCCTCTTCTTCTCATTTCG-3′. The polymerase chain reaction (PCR) thermocycler (Analytik Jena, Germany) was run with the following program;1 cycle of 94°C for 1 minute (denaturation), 57.5°C for 40 seconds (annealing), 72°C for 40 seconds (extension), followed by 30 cycles of 94°C for 40 seconds, 57.5°C for 40 seconds and 72°C for 40 seconds. A final extension was performed for 10 minutes. For the analysis of Gap-PCR products 10 µL of the amplified DNA with 2 µL of loading dye were run on 2% agarose gel (Sinaclon, Iran) and visualized by GelRed. Ladder was also run on the gels to estimate the molecular size of DNA fragments on the gel.

Ethical issues

The Ethics Committee of Zanjan University of Medical Sciences approved this study. The institutional ethical committee at Zanjan University of Medical Sciences approved all study protocols (IR.ZUMS. REC.1393.182). Accordingly, written informed consent was taken from all participants before any intervention. This study was extracted from MSc thesis of Alieh Farshbaf at this university (Thesis#A-12-33-2).

Statistical analysis

The allele frequency for each genotype could be determined as follows:

$p = [2 \times Obs(\text{Homozygote normal}) + Obs(\text{Heterozygote})]/[2 \times N]$,

$p = 1-q$,

Then the Hardy-Weinberg expectation is calculated as follows:

$Exp(\text{Homozygote normal}) = p^2 \times N$,

$Exp(\text{Homozygote variant}) = q^2 \times N$,

$Exp(\text{Heterozygote}) = 2 \times p \times q \times N$, and

The chi-square test is obtained by the following equation:

$\chi^2 = \Sigma (\text{Observed frequency} - \text{Expected frequency})^2/\text{Expected frequency}$ ($\chi^2 = 47.58$, $P < 0.001$).

Results

This study was performed on 102 HIV$^+$ and 204 healthy samples in Zanjan province in Iran. The HIV infection in the patients group was confirmed by EIA and western blot previously. In the healthy group, only one individual showed *CCR5 Δ32/Δ32* homozygote. In HIV/AIDS group, 3 heterozygotes and no *Δ32* homozygote was found. The *Δ32* allele frequency in Zanjan province in this study was 0.82% (Table 2; Figure 1).

Discussion

The major co-receptor -*CCR5*- provides natural resistance to HIV infection with 32bp deletion in both alleles (1,2). Recent studies showed gene modification and cell engineering of CCR5 can also induce resistance to HIV infection (10,13-16). Finding a suitable donor with HLA-matched by *Δ32* target genotype is one of the challenges in allogeneic transplantation (17). Despite

Table 1. Demographic information of participants in this study, in both groups (healthy and infected)

Study group	N	Gender		Mean age	Marital status	
		Male, No. (%)	Female, No. (%)		Single, No. (%)	Married, No. (%)
Healthy	204	149 (73.04%)	55 (26.96%)	34.8	92 (45.09%)	112 (54.91%)
Infected	102 HIV, n=75 (73.5%) AIDS, n=27 (26.5%)	73 (71.56%)	29 (28.44%)	36.6	36 (35.30%)	66 (64.70%)

Table 2. Distribution of Δ32 allele frequency at Zanjan province

Study group	Genotype			Total No.
	CCR5 wt/wt	*CCR5 wt/Δ32*	*CCR5 Δ32/Δ32*	
Healthy	203	-	1	204
HIV/AIDS	99	3	0	102
Total No.	302	3	1	306

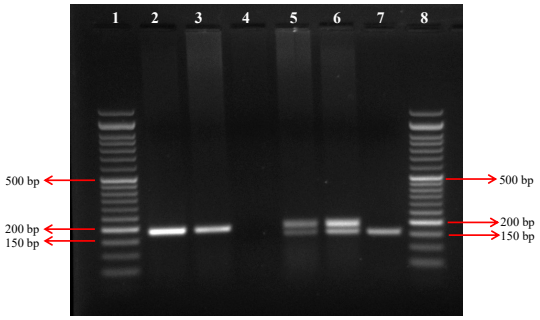

Figure 1. Gel electrophoresis of Gap-PCR amplified DNA with *CCR5Δ32* allele. Columns 1, 8 shows 50 bp DNA Ladder. Columns 2, 3 present homozygote *CCR5 wt/wt* genotype (188 bp). Column 4 is Negative control. Columns 5, 6 display heterozygote *CCR5 wt/Δ32* genotype (188bp/156 bp). Column 7 exhibit homozygote *CCR5 Δ32/Δ32* genotype (156 bp)

the successful treatment strategies for HIV, detection of *Δ32* allele frequency by scientists has progressed (18-20). Consequently, in recent therapeutic approaches, we need to find a candidate with suitable HLA-matched and target genotype, primarily. Although cord blood stem cell transplantation removed some of these restrictions, detection of target genotype still remain as a challenge (13,21).

We designed this study in order to find a candidate donor with *CCR5 Δ32/Δ32* genotype, in company with evaluation of the CCR5 *Δ32* allele frequency in Zanjan province in Iran. The results of this study exhibited *Δ32* frequency was 0.82% in 204 healthy and 102 HIV/AIDS individuals in Zanjan province. In other provinces, *Δ32* frequency has been reported in Table 3.

Distribution of *CCR5 Δ32* is different in various ethnic groups. It is demonstrated that north European countries such as Finland, Sweden and Iceland have the highest *CCR5 Δ32* frequency with 14-16% and this rate is reduced gradually from North to South. In the central and west regions, *Δ32* frequency reaches 10% and in the south region is reported about 4-6% (22).

Similar to the other Middle-East countries, Iran has low frequency in *Δ32* allele. The main reason of differences in *Δ32* allele frequency between Iranian and European population is because of different climate-geographical condition, migration, genetic admixture and positive natural selection (23-26). In the present experiment, we briefly explain the effects of these factors on distribution of *Δ32* allele frequency. As we mentioned already, one of the causes of different *Δ32* allele frequency between Iranian and European populations is genetic admixture of Iranian with dissimilar people following historical events. Europeans mostly mixed with similar populations (23, 24). In addition, *Δ32* allele was one of the survival factors for epidemic infections in 14th and 17th centuries. During those times, epidemic smallpox killed more than 30% of the European population. In the process of virulence, the causative agent of smallpox -Variola- enters into immune cells by chemokine receptors and *CCR5 Δ32* was one of the resistance factors for patients affected by smallpox. Besides, recent studies demonstrated the effect of CCR5 variation on other viral infections such as coronavirus, Rocio virus, Zika virus, Epstein-Barr virus and Rhinovirus (16,27). Hence detection of CCR5 variants like *Δ32* can help us provide a permanent cure and vaccine development in viral infection.

Table 3. Frequency of *CCR5 Δ32* allele in provinces of Iran

Provinces		N (Healthy)	N (HIV)	CCR5 *wt/wt*	CCR5 *wt/Δ32*	CCR5 *Δ32/Δ32*	*Δ32* Frq.%	*wt/Δ32* Frq.%	Ref.
Fars		395	-	384	11	0	1.4%	2.8%	(23)
Uremia		190	-	186	4	0	1.05%	2.1%	(30)
13 Provinces	Gilan	20		19	1	0	0.57%	1.1%	(24)
	East Azerbaijan	50		49	1	0			
	West Azerbaijan	50		49	1	0			
	Qazvin	45		44	1	0			
	Tehran	100	40	97	2	1			
	Semnan	30		30	0	0			
	Kurdistan	35		35	0	0			
	Qom	30		30	0	0			
	Isfahan	40		40	0	0			
	Khorasan	40		40	0	0			
	Yazd	30		30	0	0			
	Lorestan	30		30	0	0			
	Hormozgan	30		30	0	0			
Zanjan		204	102	302	3	1	0.82%		Present study

Conclusion

In this study, we detected only one *CCR5 Δ32* homozygote. Based on the key role of *CCR5Δ32* in HIV therapy, individuals with target genotype can donate their cord blood or hematopoietic stem cells to HIV infected patients, resulting in an effective cure. Cord blood stem cell donations with the target genotype reduce restrictions of allogeneic SCT like HLA-matching between donor and recipients (7, 13, 14, 21).

Sorting the cord blood and hematopoietic stem cells from one person that detected in this study can provide cell therapy with target genotype *(Δ32/Δ32)* for HIV infected patient's treatment, even by different ethnic groups. Although an increase in sample population can detect more CCR5 *Δ32* genotype in future studies, HLA-matching is not very important in contrast to allogeneic SCT (13, 21) and CCR5 *Δ32* genotype is low-prevalence in Iran and Middle-East countries (28).

Our study confirms previous reports that Iranian populations compared to Europeans are more susceptible to HIV infection like Arabs and Middle-East population (28,29). Since, the variants in their genetic background especially *CCR5* gene provide susceptibility or resistance to infection (23, 24).

In addition, family of our target genotype can present more *Δ32* genotypes, homozygote or heterozygote. A group sample of these families can be collected and be suited regarding susceptibility to immune or non-immune disease in comparison to other groups. The results can reveal the association of the disease with target genotype in various ethnic groups.

Limitations of the study

- Refusal of HIV/AIDS infected patients to donor their blood sample
- According to the of restricted budget we could not study more related genes

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Authors' contribution

AF conducted the experiment and prepared the main manuscript. AB interpreted the data and helped in supervising the study and manuscript edition. SF was responsible for statistical analysis. AE designed and supervised the study and developed the concept.

Conflicts of interest

None of the authors report conflicts of interest relevant to this study.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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